ISBT/ICSH International Workshops and Proficiency Test on Molecular Blood Group Genotyping

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Overview of Talk

- 2004 Workshop
  - Organization & outcome
- 2005 Quality Asssurance Exercise
  - Organization & Results
- 2006 Workshop
  - Organization & outcome
- Future

2004 First ISBT/ICSH International Workshop on Blood Group Genotyping: Beginnings

- Identified a need for workshops for people who were interested in molecular analyses for blood groups
- Organizers
  - Geoff Daniels, Bristol, UK
  - Martin Olsson, Lund, Sweden
  - Ellen van der Schoot, Amsterdam, The Netherlands
- Call for interest in participation
- Handling fee charged

2004 ISBT/ICSH International Workshop on Blood Group Genotyping

- 30 laboratories participated
- Six samples were distributed
  - 2 represented DNA from transfusion-dependent patients for testing for multiple polymorphisms
  - 2 represented fetal DNA prepared from amniotic fluid for RhD, Rhc, and K testing
  - 2 represented plasma from RhD-negative pregnant women for fetal RhD testing
- 20 laboratories were represented at the feedback meeting in Edinburgh, Scotland

2004 ISBT/ICSH International Workshop on Blood Group Genotyping: Results

- A variety of techniques were used
- Errors were obtained in
  - ABO: 3
  - Rh: D 16; C/c 5; E/e 2
  - S/s: 1; Kell: 5; Fy: 1; Jk 1
- Several errors were clerical
- Some D and Fy errors were due to not testing for silencing SNPs
- For details see
  - Daniels et al. Vox Sang 2005;88:136-142
  - www.blood.co.uk/research

2004 ISBT/ICSH International Workshop on Blood Group Genotyping: Recommendations

- **Appropriate controls should be included**
  - **a no-template (water) control, at least as a batch control**
  - **at least one negative control**
  - **at least one positive control**
  - **a positive control for input DNA involving consensus PCR in the same tube (multiplex). When multiplex PCR is not possible and or consensus PCR is performed in a separate tube, genotyping PCR performed at least in duplicate**

2004 ISBT/ICSH International Workshop on Blood Group Genotyping: Recommendations (continued)

- RhD testing should include a test for *RHDΨ*
- RhC/c testing should not be dependent on the nucleotide 48 polymorphism
- Duffy testing should include a test for the GATA mutation
- *RHD* zygosity testing should involve a method that determines the quantity of *RHD* genes present relative to a gene of known zygosity, and should include a test for *RHDΨ*
- When no paternally-derived fetal marker is detected in tests on maternal plasma, a fetal RhD-negative result should be reported with a caveat that it had not been possible to include all appropriate controls
- Numerical nomenclature should be considered for blood group antigens/alleles, such as *KEL1* or KEL*1 and *KEL2* or KEL*2 instead of or in addition to *K* and *k*, respectively, to avoid simple reporting errors

2004 ISBT/ICSH International Workshop on Blood Group Genotyping: Outcome

- It was agreed at the feedback meeting that the workshop was a useful exercise and that workshops will take place every two years, with a feedback meeting at the ISBT Congress
- The next workshop, of similar format, will be (and was) organized in 2006 by Geoff Daniels, Ellen van der Schoot, and Martin Olsson, with the feedback meeting in Cape Town in September of 2006 to be arranged by Elizabeth Smart
- In addition, in order to provide an annual external quality assurance scheme, a more limited exercise, involving two blood or DNA samples and no feedback meeting, will take place in the intervening years. Marion Reid at the New York Blood Center will (and did) arrange the first, in 2005.

2005 ISBT/ICSH Quality Assurance Exercise

- 29 laboratories participated
- No fee was charged but participating laboratories paid for shipment of samples
- DNA samples from 2 donors who had been fully phenotyped and tested for clinically relevant SNPs were distributed
- Results were collated by Geoff Daniels

2005 Quality Assurance Exercise: Results

- A total of 496 tests were performed for polymorphisms in ABO, MN, Ss, RhD, C, c, E, e (including *RHDΨ* and *RHD* zygosity), K/k, $Fy^a/Fy^b/Fy/Fy^x$, Jk^a/Jk^b, Do^a/Do^b
- There were only 3 incorrect results
  - S–s+ instead of S+s–
  - M+N+ instead of M+N–
  - Weak D instead of D+
- For details see www.blood.co.uk/research

2006 Second ISBT/ICSH International Workshop on Blood Group Genotyping

- 41 laboratories participated including from
  - Argentina, Australia, Brazil, Canada, China, Europe, USA
- Six samples were distributed
  - 2 represented DNA from transfusion-dependent patients for testing for multiple polymorphisms
    - Tested by 38 labs
  - 2 represented fetal DNA prepared from amniotic fluid for RhD, Rhc, and K testing
    - Tested by 39 labs
  - 2 represented plasma from RhD-negative pregnant women for fetal RhD testing
    - Tested by 20 labs
- Many laboratories were represented at the meeting in Cape Town, South Africa

2006 ISBT/ICSH International Workshop on Blood Group Genotyping: Results

- A variety of techniques were used
- Errors were obtained in
  - Sample 1: 34 errors (most in Rh) D, Cc, MN, Ss, Jk, Do
  - Sample 2: 6 errors D, Cc, Kk, Fy
  - Sample 3: 8 errors D, Cc, Kk
  - Sample 4: 2 errors D, Cc
  - Sample 5: 1 error
  - Sample 6: 1 error
- Several errors were still clerical

2006 ISBT/ICSH International Workshop on Blood Group Genotyping: Techniques

Variety of techniques; greater than in 2004:

- **Included multiplex PCR, PCR-RFLP, PCR-AS, Real-Time PCR, pyrosquencing, and microarray**
- **Most labs have a favorite method**
  - **Multiplex PCR for *RHD***
    - **Exons 4 & 7 or all *RHD* specific exons depending on reason for testing**
  - **Trend is towards Real-Time PCR**

Origin of techniques:

- **Literature**
- **In-house**
- **Commercial kits**
- **Usually a combination**

2006 ISBT/ICSH International Workshop on Blood Group Genotyping: Implementation of 2004 Recommendations

- **Appropriate controls should be included**
  - **a no-template (water) control, at least as a batch control: included by most labs**
  - **at least one negative control: used by most labs**
  - **at least one positive control: used by most labs**
  - **a positive control for input DNA involving consensus PCR in the same tube (multiplex): used by most labs**

2006 ISBT/ICSH International Workshop on Blood Group Genotyping: Implementation of 2004 Recommendations

- RhD testing should include a test for *RHDΨ*: used by most labs
- RhC/c testing should not be dependent on the nucleotide 48 polymorphism: all labs tested for *RHCE* intron 2 polymorphism
- Duffy testing should include a test for the GATA mutation: used by most labs
- When no paternally-derived fetal marker is detected in tests on maternal plasma, a fetal RhD-negative result should be reported with a caveat that it had not been possible to include all appropriate controls: 6 labs mentioned use of controls for presence of fetal DNA
- Numerical nomenclature should be considered for blood group antigens/alleles, such as *KEL1* or KEL*1 and *KEL2* or KEL*2 instead of or in addition to *K* and *k*, respectively, to avoid simple reporting errors: implemented by some labs

2006 ISBT/ICSH International Workshop on Blood Group Genotyping: Cell-free Fetal DNA

- **2 plasma samples, week 16-18 gestation**
  - one RhD+; one RhD–; both from a male fetus
  - 19 of 20 labs obtained the correct result
  - 15 labs tested for presence of Y chromosome
  - Consensus: samples for next workshop should be more difficult!
- **Tests offered routinely**
  - 15 labs offer *RHD* determination
  - 5 labs offer *RHC/c* determination
  - 3 labs offer *RHE/e* determination
  - 3 labs offer *KEL1/2* determination (difficult assay)

2006 ISBT/ICSH International Workshop on Blood Group Genotyping: Cell-free Fetal DNA (continued)

- **Plasma volume used for assay:**
  - Range of 200µl to 1000µl
    - average 800µl; some used only 200µl
- **DNA extraction (manual or kit):**
  - Subtle differences in quantity of DNA recovered (some DNA lost on columns); may cause loss of sensitivity in samples with low levels of fetal DNA
  - Continued development for tests that can be used for samples with low levels of fetal DNA
- **Future:**
  - Need for standardization: plasma volume used; DNA isolation method
  - Standards to control effectiveness of DNA extraction and genomic DNA standards to control PCR specificity (e.g., 50pg DNA/mL is approximately 10 genome equivalents)

2006 ISBT/ICSH International Workshop on Blood Group Genotyping: Discussion Points

Mainly concerned standards and standardization:

- **Terminology for reporting of genotyping results and alleles: almost as many versions as participating labs**
  - **Being addressed by ISBT Terminology Committee**
- **Need genomic standards**
  - **Half the participants were using in-house controls**
  - **CE-marked standards (cultured cell lines) available for HLA assays; do similar for red cell antigens**
  - **Need to define "normal"; then consider establishing cell lines**
  - **How to control variants; sharing of resources**
  - **NIBS workshop in March will focus on these issues; but "users" need to decide on composition of standard panels**

2007 ISBT/ICSH Quality Assurance Exercise

- **Decision:**
  - **Those wishing External Quality Assurance (EQA) for SNP testing can subscribe to the exercise prepared for the German Society of Transfusion Medicine and Immunohematology (since 1998) by an experienced non-profit organization (INSTAND) in Germany**
  - **Possibility of 2 exercises per year at the cost of 30 Euro**
  - **Those wishing plasma samples for cell-free fetal DNA can subscribe to the European SAFE testing workshop**

2006 ISBT/ICSH International Workshop on Blood Group Genotyping: Discussion Points (continued)

Future workshops:

- **Overwhelming decision that there should be a 3rd workshop in 2008**
- **Consider applying a strict deadline to receipt of results if labs are using the workshop for EQA**
- **Level of difficulty and type of samples:**
  - **Should include "regular" sample(s) to serve as EQA and "brain-teaser" sample(s) with the option not to test**
  - **To again include plasma samples for cell-free fetal DNA**

ISBT/ICSH International Workshop on Blood Group Genotyping: Purpose of Workshops

- To provide external quality assurance for laboratories that provide a diagnostic molecular blood group genotyping service
- Communication between those laboratories worldwide
- The workshops will be continued to be held every two years with a feedback meeting at the ISBT International Congress
- A smaller QA exercise, with no feedback meeting, during the interim years